APJBG

Incidence of piperacillin-specific drug antibody in patients with and without piperacillin

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ABSTRACT

The aim of this study was to investigate the incidence of piperacillin-specific drug antibody in patients with and without piperacillin. Piperacillin antibody was detected by piperacillin-induced hemolysis test kit. Three hundred samples were collected from patients who had been treated with piperacillin within 3 months; and 222 samples were from patients who were treated with other antibiotics; and a normal group of 120 samples was from healthy blood donors. The results showed that the positive rates in the piperacillin group, the other antibiotic group and the control group were 11.33%, 4.95% and 1.67%, respectively, which was statistically significant. The antibody titer was from 2 to 64. The antibody titer of the piperacillin group was significantly higher than that of the other two groups. The study suggests that piperacillin is able to induce piperacillin-specific drug antibody and drug antibody detection is useful for maintaining the safety of medication.

Keywords: piperacillin-specific drug antibody, piperacillin sensitized RBC, drug safety

INTRODUCTION

The top five rankings of China´s adverse drug re−action monitoring reports in 2017 were antimicrobi−als, cardiovascular drugs, antineoplastic agents, wa−ter-electrolyte, acid-base balance drugs and digestive system drugs. The reported proportion of antimicro−bial drugs was highest (43.9%). The top 3 varieties of serious adverse reactions / incident reports were all antimicrobials[1]. Chinese domestic and wider international adverse drug reaction / incident report forms however, only focus on the causality and se−verity of side effects caused by drugs, and diagnostic tools and preventive measures for laboratory diag−nostic drug antibodies are still lacking[2].

Piperacillin (a semi-synthetic penicillin) is used to treat respiratory tract, gastrointestinal tract and urinary tract infections, as its suitability for treating sensitive strains of certain gram-negative bacteria and anaerobic bacteria[3−4]. In the past half century, the use of pipera−cillin at home and abroad has reached the top five of all antibacterial penicillin injections.

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Piperacillin is the third most common drug that causes drug-induced hemolytic anemia, of which cefotetan is the most common and ceftriaxone is the second one [5]. The mechanism of piperacillin−induced immune hemolytic anemia is an antibody/hapten immune response, in which piperacillin binds to surface protein of erythrocyte-cell membranes to form the hapten/protein complex. Penicillin-induced hemolysis, which usually takes place in the ex−travascular space, usually occurs approximately 10 days after penicillin treatment[6−7]. In this study, the piperacillin induced hemolysis kit was used to ana−lyze the incidence of piperacillin antibody in patients with and without piperacillin.

MATERIALS AND METHODS

Blood samples

A total of 642 blood samples were collected from the Sir Run Run Shaw Hospital affiliated to Zhe−jiang University from January 2017 to May 2018, including 300 samples from patients who had been treated with piperacillin within 3 months (piperacil−lin group), 222 samples from patients who were treated with other antibiotics (other antibiotic group) and the normal group of 120 samples from healthy blood donors (control group). Whole blood was taken and plasma was separated for testing. The penicillin skin test was negative before the patient was given the drug. The study was approved by the Human Research Ethics Committee of the hospital and had informed consent from the patients them−selves.

Drug antibody detection

A piperacillin-induced hemolysis test kit was used as the experimental reagent (Jiangsu Zhongji Wantai Bio-Pharmaceutical Co., Ltd., batch num−ber 1803002, 1803004, 18040017, 18050038, 18060025). The main components of the kit were drug-sensitized red blood cells, micro-column gel cards containing anti-human IgG special for drug antibody detection and positive and negative control (where the manufacturer provides the sample with or without piperacillin antibody).

According to the test kit´s instruction manual, the plasma to be tested was diluted 1:19 with physi−ological saline. Each test consisted of: ① Positive control: positive control 50 μL + drug-sensitized red blood cells 25 μL; ② Negative control: negative control 50 μL + drug-sensitized red blood cells 25 μL; ③ Self-control: Plasma to be tested 50 μL +un−sensitized red blood cells 25 μL; ④ Drug antibody detection: Plasma to be tested 50 μL+drug-sensitized red blood cells 25 μL.

Further instructions were to: take the drug antibody detection reagent card, add the cells and plasma re−spectively, mix well, incubate for 1 h at (37 ± 1)℃ in a reagent card incubator and then centrifuge for 5 min （900 r/min×2 min and 1,500 r/min×3 min）. The results were observed and recorded within 30 min.

Statistical analysis

All the statistical analyses were conducted by us−ing SPSS version 19.0. Comparison results with a 2-sided. *P* value < 0.05 were considered statistically significant.

RESULTS

Piperacillin antibody detection

The positive rates of piperacillin antibody were: 34 cases in the piperacillin group: 11.33% (34/300); 11 cases in the other antibiotics group: 4.95% (11/222); and 2 cases in the normal control group: 1.7% (2/120,**Table 1**).

Table 1 The detection of piperacillin antibody

Groups	n	Positive (n)	Weak positive(n)	Negative (n)	Positive rate(%)	P value
Piperacillin group	300	20	14	266	11.33	0.008
Other antibiotic group	222	4	7	211	4.95	
Control group	120	0	2	118	1.67	

Piperacillin antibody titer

The antibody titers of all 47 positive samples were determined(**Table 2**). In the piperacillin group, there were 14 samples with an antibody titer of less than 2, 8 samples with an antibody titer of 2, 4 samples with titer of 4, 3 samples with titer of 8, 2 samples with titer of 16, 2 samples with titer of 32 and 1 sample with a titer of 64, respectively; In the other antibiotic group, there were 7 samples with an antibody titer of less than 2, 3 samples with antibody titer of 2, and 1 samples with an antibody titer of 4, respectively. In the control group there were only 2 samples with an antibody titer less than 2.

Age analysis

The observation objects´ ages were analyzed separately. In piperacillin group, the positive anti−body rates of 18~55 age and > 55 age were 10.6% and 11.9%, respectively; in other antibiotic group,

Table 2 **The titers of piperacillin antibody** (*n*)

Groups	Drug antibody titer of piperacillin						
	Total	< 2	2	4	8	16	32	64
Piperacillin group	34	14	8	4	3	2	2	1
Other antibiotic group	11	7	3	1	0	0	0	0
Control group	2	2	0	0	0	0	0	0

the antibody positive rates of 18~55 age and > 55 age were 3.9% and 5.8%, respectively. No blood donors over 55 years old were in control group (***Table 3***).

Table 3 **Sample age statistics**

Groups	*n*	Positive (*n*)	Weak positive(*n*)	Negative (*n*)	Positive rate(%)	*P* value
Piperacillin group	300	20	14	266	11.33	0.008
Other antibiotic group	222	4	7	211	4.95	
Control group	120	0	2	118	1.67	

DISCUSSION

In 2017, the National Adverse Drug Reaction Monitoring Network received 433,000 new and serious adverse drug reaction / incident reports, of which 55,000 were serious. Among the suspected drugs, the first three categories of chemical cases were anti-infective drugs (42.3%), cardiovascular system drugs (10.0%), and cancer treatment drugs (7.3%), respectively. Anti-infective drugs were by far the main cause of adverse drug reactions [1]. The current monitoring system for adverse drug reactions mainly observes and tests the severity of adverse reactions and looks for the causes, but lacks direct laboratory diagnostic tools, and therefore lacks preventive methods [2].

Drug-induced immune hemolysis can be caused by different mechanisms. It is generally believed that there are three main mechanisms: ① The drug is converted into a complete antigen after binding to red blood cells, and the whole immunogenicity is expressed, and the immune cells recognize the drug antigen to destroy the cells; ② Antibodies in plasma and drugs form immune complexes that are adsorbed on cells and produce antibodies against drugs or their metabolites; ③ The drug only binds loosely to the cells, and the cell does not produce drug antibodies, but produces autoantibodies. A penicillin-like drug represented by piperacillin, which induces drug antibody production, has a hapten mechanism. Piperacillin as a hapten covalently binds to proteins on the erythrocyte membrane, stimulating the body to produce antibodies. After the production of antibodies, the continued use of large amounts of drugs leads to the production of drug-induced immune hemolysis. [8–11]

Leger RM *et al.*[12] used piperacillin-sensitized red blood cells to detect the plasma of patients, found a certain proportion of blood donor plasma and patient plasma agglutinated with piperacillin-sensitized red blood cells, and piperacillin antibody was detected in some patient plasma. In China, domestic research on drug antibodies is in its infancy, and lacks a systematic approach. According to one report, 8 cases of anti-penicillin antibodies were detected in 181 samples, among which 4 cases had obvious hemolytic reaction. In addition, another report published data from 327 antibiotic drug treatments, showing 13 cases having anti-drug antibodies, 4 of which had an obvious hemolytic reaction [13–14].

In this study, the piperacillin induced hemolysis kit was used to detect piperacillin antibody in patients´ plasma by using drug sensitized erythrocyte in microcolumn gel. Among them, 120 participants represented healthy blood donors, from which the positive antibody rate was 1.7%; 222 cases of patients were treated with other antibiotics, resulting in a positive rate of 4.9%; 300 patients were treated with piperacillin, at a positive rate of 11.3%. The finding was that drug antibodies produced after drug treatment may increase the risk of adverse reactions to subsequent antibiotic treatment. In the case of adverse reactions, false negative cases is common: the penicillin skin test is negative, but there remains a large number of drug antibodies in patients plasma. When a patient see a doctor, the clinician should ask about the history of allergies and family history in detail. When using piperacillin, skin tests and drug antibody tests should be performed at the same time, which can effectively reduce adverse reactions caused by piperacillin.

The antibody titer of the piperacillin group was significantly higher than that of the other groups. There were 8 cases in which the antibody titer of the piperacillin group was more than 8, but the antibody titers of the other two groups were all less. Positive results in other antibiotic groups may be due to drug cross-reactivity. Piperacillin belongs to the penicillin drug group. Antibiotic drugs have similar molecular structures and their immunogenicity is relatively close, so cross-reaction may occur between drug antibodies and drugs. The positive result in the control group may have been due to the changes in the erythrocyte membrane caused by piperacillin, and the non-immune adsorption of protein on the erythrocyte membrane, causing the test results to be positive, so the titer was relatively low.

In this study, statistical analysis was performed

on 642 samples. The results indicate that as patients age increases, the likelihood of using antibiotics in–creases, and subsequently the incidence of drug anti–bodies also increases. It is possible that only the first piperacillin is sensitized, or that a large quantity of piperacillin will activate the immune cells to produce a large number of piperacillin antibodies, leading to serious hemolysis and serious drug-induced im–mune hemolytic anemia. This means that different individuals will have different antibody piperacillin titers. Piperacillin is commonly used in patients with cystic fibrosis screening, however anti-piperacillin antibodies should also be taken into consideration whenever these patients develop hemolytic anemia or test positive in the direct antiglobulin test.

Commonly used drug-based antibody detection methods include the traditional test tube method, micro-column gel method, ELISA method, flow cytometry, and so on. The microcolumn gel anti-human globulin method is widely used in the de–tection of irregular blood group antibodies. The method for the detection of drug antibodies was established by combining the erythrocyte sensitiz–ing technique with the microcolumn gel detection system. This can effectively detect piperacillin drug antibodies in blood samples and provide a judgment basis for clinical treatment and the safe use of transfused blood.

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